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Hybridoma. 1986 Jul;5 Suppl 1:S117-23.

Med Oncol Tumor Pharmacother. 1986;3(3-4):141-6.

Hybridoma. 1986 Jul;5 Suppl 1:S163-70.

Hybridoma. 1986 Jul;5 Suppl 1:S151-61

Hybridoma. 1986 Jul;5 Suppl 1:S125-32.

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Treatment of Patients with Metastasizing Colo-rectal Carcinoma with Mouse Monoclonal Antibodies (Moab 17-1A): A Progress Report

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ABSTRACT

Eight patients with metastasizing colo-rectal carcinoma have been treated with Moab 17-1A. Before infusion the antibodies were incubated *in vitro* with isolated autologous blood mononuclear cells (AMC) enriched for monocytes/macrophages. Treatment was given in repeated courses (2-4 times) up to a maximum amount of 1000 mg Moab 17-1A. Two patients had an objective tumor reduction. In further four patients a period of stable disease varying between 3-6 months was observed. Therapy was well tolerated. Out of 24 treatment courses only on one occasion an anaphylactoid reaction occurred at the third infusion. All patients developed anti-mouse antibodies of IgG and IgM class with increasing levels during the course of therapy. Repeated tumor biopsies and immunohistochemical analyses showed no antigenic modulation, a weak staining for mouse IgG, no deposits of complement components but no obvious increases in the number of cells infiltrating the tumors 24 h after infusion of antibody-armed AMC.

INTRODUCTION

Tumor cells may carry antigenic surface membrane structures which distinguish them from their non-malignant counterpart. Such antigens can be exploited not only for diagnostic use but also as targets for immunotherapy. Tumor-specific antigens have so far only been identified in experimental tumors. More often, tumor antigens are present not only on malignant cells but can also be detected on normal cells. Such tumor-associated antigens (TAA) may represent normal differentiation antigens or viral antigens. These may be abundant on fetal cells, non-detectable or sparsely expressed on normal cells, but re-expressed, often in high concentrations, on tumor cells.

Specific antibodies can be raised against TAA by the mouse hybridoma technology. Such antibodies when infused into a tumor bearing patient may bind to the tumor cell surface. Subsequent activation of immunological defence mechanisms may eventually lead to tumor cell destruction.

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Specific mouse monoclonal antibodies have been produced in large amounts against a differentiation antigen on colo-rectal tumor cells called 17-1A^(1,2) and have been used by us in a pilot study to treat patients with metastasizing colo-rectal cancer.

The in vivo mechanisms of Moab mediated tumor cell destruction are not fully known. One effector function may be the ADCC-reaction (antibody dependent cellular cytotoxicity). It has been suggested that effector cells of importance in the tumor cell killing might be monocytes/macrophages and NK (natural killer)-cells. In the present treatment protocol we have taken advantage of this suggestion and isolate blood AMC and enrich for monocytes/macrophages which are incubated in vitro with Moab 17-1A. The armed mononuclear cells (and unbound Moab 17-1A) are then infused into the patient with the aim to increase the numbers of cytotoxic cells in the tumor lesions. This report is a preliminary progress communication of a study with the purpose to analyse the therapeutic effect of Moab 17-1A and certain immune reactions related to therapy.

MATERIALS AND METHODS

Patients

Eight patients have entered the study (Table 1). All have had surgery with removal of the primary tumor. In three patients (no:s 4,5,6) metastases were diagnosed at operation while in the other patients the metastases developed later during the course of the disease. The first two patients (no:s 1,2) did not fulfil the inclusion criteria later applied. Both had a considerable tumor volume and pat. no 1 had previous chemotherapy. After that the following inclusion criteria were used: a small but measurable tumor volume; a good general condition (Karnofsky index of 100%); no complicating disease and no previous chemotherapy or radiation treatment. The presence of tumor cells in a metastasis was always histologically confirmed.

Clinical follow-up

Before therapy a careful clinical examination was made. The following laboratory tests were performed: ESR, Hb, WBC, platelet counts, liver function tests, s-creatinin, s-electrolytes, s-protein electrophoresis including immunoglobulin quantitation and serum complement components (C3, C3d, C4) conc., serum tumor markers (CEA, CA19-9) and urine analysis for glucose, protein and microscopic examination. An X-ray of the lung was done as well as computed tomography and ultrasonic examination of the abdomen and immunoscintigraphy using radio-labelled monoclonal antibodies against CEA and 17-1A. Blood and urine analyses were repeated every week. X-ray of the lungs and ultrasonic examination of the abdomen were made once a month. Computed tomography of the abdomen was used to confirm tumor reduction observed by ultrasound.

Isolation of autologous mononuclear cells (AMC) and binding of Moab 17-1A to the cells.

An IBM 2997 blood cell separator was used to extract about 450 ml of a monocyte/lymphocyte cell concentrate. The cell suspension was then centrifuged twice on Lymphoprep gradient (Nyegaard, Oslo, Norway) using an IBM 2991 blood cell processor and washed three times in BSS. The concentrate was diluted in BSS containing 1% human serum albumin to a final volume of 225 ml. The cell suspension was then incubated at room temperature for one hour on a cradle with the total amount of Moab 17-1A to be infused.

Treatment protocol

400 mg of Moab 17-1A together with AMC was given at the first infusion time.

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TABLE 1. PATIENT CHARACTERISTICS

Pat. no	Sex/age	Prim. site	Dukes class	Time from prim. surg. (months)	Metastases	Tumor cells expressing 17-1A anti- gen	Prior therapy
1.	F/37y	Rect.	C2	24	Liver Lung Pelvis	+	Chemo- therapy (2 courses of MFL)
2.	F/69y	Rect.	B3	25	Liver Lung Stomi	+	None
3.	M/70y	Rect.	C3	9	Liver Lung Pelvis Stomi	Necrotic material	None
4.	M/32y	Col.	C3	1	Mesente- rial root	ND	None
5.	F/48y	Rect.	D	3	Liver	+	None
6.	F/55y	Col.	D	1	Liver	+	None
7.	M/75y	Col.	C2	36	Liver	+	None
8.	F/81y	Col.	C2	75	Liver	+	None

F=female; M=male; MFL=methotrexate, 5-fluoro-uracil, leucovorin; ND=not done

At the following courses 200 mg of Moab 17-1A together with AMC were given. The infusion period was 30-60 min. A total amount of 1000 mg Moab 17-1A during four courses was intended to be given unless there was evidence of disease progression or allergic reactions. The interval between the courses was six weeks. Before every infusion an intracutaneous test with Moab 17-1A was made. The test was read after 4 h.

Mononuclear cell surface markers

Mononuclear cell subpopulations in suspension were determined by indirect immunofluorescence as described earlier⁽⁵⁾. The following monoclonal antibodies were used in the first step: OKT3 (Pan-T), OKT4 (helper/inducer-T), OKT8 (suppressor/cytotoxic T), OKM1 (monocytes/macrophages) (Ortho Pharmaceutical Raritan, N.J., USA); Leu M-1, Leu M-2, Leu M-3, Leu M-5 (monocytes/macrophages); Leu-7, Leu-11b (NK-cells) (Becton-Dickinson, Mountain-View, CA, USA); B1 (B-lymphocytes) (Coulter Elec. Ltd., USA). In the second step absorbed goat anti-mouse IgG (Meloy, Springfield, VA, USA) was used. 200-400 cells were counted in a Leitz Dialux 20 B with epi-illumination in ultraviolet light x 1000 magnification.

To study the binding of Moab 17-1A to the cell surface of AMC a sample from the cellconcentrate after incubation in vitro was washed in Hank-Tris solution and incubated with the fluoresceinated goat anti-mouse IgG conjugate.

ELISA

Mouse immunoglobulin and class specific human anti-mouse antibodies were assayed in ELISA using flat-bottom micro-titer ELISA plates (Dynatech) which

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were coated at 37° C over night with goat anti-mouse IgG (Nordic, Tilburg, The Netherlands) for mouse Ig determination and with mouse Ig (Nordic, Tilburg, The Netherlands) for human anti-mouse Ig analyses.

After blocking with 0.5% bovine serum albumin in coating buffer for 1 h at 37° C, diluted samples to be tested were added in triplicate for 1.5 h at 37° C and finally plates were reacted for 1.5 h at 37° C with goat anti-mouse IgG peroxidase conjugate (Nordic, Tilburg, The Netherlands) or rabbit anti-human IgG, IgM, IgE peroxidase conjugate (DAKO A/S, Copenhagen, Denmark) respectively as indicated above. After enzyme reaction for 0.5 h using 1.2-phenylen-diamindihydrochlorid (0.55 mg/ml) in TRIS-HCl buffer pH 7.6 containing 30 µl H₂O₂ per 100 ml of volume, the extinction at 450 nm was measured using an automatic ELISA reader (Titertec, Multiscan). Absorbance of normal control serum for IgG anti-mouse antibodies was < 0.15 and for IgM antibodies < 0.03. Background, determined by incubating the antigen-coated wells with medium alone, was subtracted.

Immunohistopathological analyses

Thick needle biopsies guided by ultrasound and fine needle aspiration cytology was obtained from metastatic tumor lesions. The biopsies were transferred to physiological saline. Part of the material was fixed in formal sublimate (B5) for routine histopathology and the rest was quick-frozen in liquid nitrogen and stored at -70° C. Cryostat sections were fixed in -20° C acetone for 10 min. Tumor cell antigens were also assayed on fixed material. The following Moab against tumor cell antigens were used: 17-1A, 19-9A, 19-9B, Ga73-3 (prof. H. Koprowski, Wistar Inst. Phil., USA); Leu-2 (suppressor/cytotoxic-T), Leu-3 (inducer/helper-T), Leu-4 (pan-T) (Becton-Dickinson); anti-cytokeratin (Labsystems, Helsinki, Finland). A FITC conjugated sheep antiserum against complement factor C3 was obtained from Wellcome, Beckenham, England. For detection of primary Moab either a mouse peroxidase-antiperoxidase (PAP) or a three step alkaline phosphatase (AP) method was used. The second and third step reagents (rabbit anti-mouse Ig, mouse-PAP, AP conjugated rabbit anti-mouse Ig and AP conjugated swine anti-rabbit Ig) were obtained from Dakopatts A/S. Appropriate dilutions of Moabs, second and third step reagents were incubated for 30 min. at room temp. with intervening repeated 10 min. rinses in Tris buffered saline. In the PAP staining the antibody binding was visualised using diaminobenzidine(DAB), whereas in the AP staining an alkaline phosphatase substrate kit "Vector red" from Vector Lab. Burlingame, USA was used. For detection of C3, direct IFL with a Zeiss epifluorescence microscope was used.

The frequency of tumor cells positively stained with the Moabs were counted in randomly chosen high power fields (HPF = x 40). The number of infiltrating positively stained leucocytes was estimated by counting cells in ten randomly chosen HPFs. Since some of the Moabs, Leu M-1, Leu-11b, crossreacted with tumor cells, only cells located outside the tumor glands were counted.

RESULTS

In the morning the day of treatment a leucapheresis was performed. The mean total number of cells obtained from peripheral blood from 24 cellaphereses was 8.1×10^9 cells (range $2.0-21.5 \times 10^9$). The various mononuclear cell subpopulations found in the cell concentrates are shown in Table 2.

After incubation with 400 mg Moab 17-1A 36% cells (range 20.5-49.0%) carried mouse Ig on the cell surface and the corresponding figures after incubation with 200 mg were 39% positive cells (range 18.5-61.0%).

Before administration of Moab 17-1A an intracutaneous testing with Moab 17-1A was done. In pat. no 2 a positive reaction was noted at the second infusion time. Before the intravenous administration of Moab the patient received 200 mg hydrocortison. She only experienced some palmitch at the end of the infusion period. In pat. no 8 at the third treatment time the reaction was misread as negative but was obviously positive. She developed an anaphylactoid reaction with hypotension within 5 min. from start. After immediate stop of the infusion and an

mouse IgG (Nordic, Tilburg, The
mouse Ig (Nordic, Tilburg, The

in coating buffer for 1 h
in triplicate for 1.5 h at
it 37° C with goat anti-mouse
therlands) or rabbit anti-
S, Copenhagen, Denmark) res-
on for 0.5 h using 1.2-phe-
Cl buffer pH 7.6 containing
t 450 nm was measured using
Absorbance of normal control
d for IgM antibodies < 0.03.
coated wells with medium alone,

fine needle aspiration cy-
he biopsies were transferred to
ed in formol sublimate (BS)
frozen in liquid nitrogen
in -20° C acetone for 10 min.
erial. The following Moab
A, 19-9B, Ga73-3 (prof. H.
ssor/cytotoxic-T), Leu-3 (in-
anti-cytokeratin (Labsystems,
rum against complement factor
For detection of primary Moab
three step alkaline phos-
step reagents (rabbit anti-
se Ig and AP conjugated swine
appropriate dilutions of Moabs,
0 min. at room temp. with
d saline. In the PAP stain-
nobenzidine(DAB), whereas in
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TABLE 2. MONONUCLEAR CELL SUBPOPULATIONS (%) IN CELL CONCENTRATES* FROM 24 CELLAPHERESSES

Subpopulations identi- fied by monoclonal antibodies	Mean	SEM (±)	Range
OKM 1	30.4	3.9	2.8 - 66.0
Leu M-1	15.3	4.8	2.5 - 49.5
Leu M-2	22.1	5.4	3.5 - 49.0
Leu M-3	23.7	4.1	5.0 - 41.5
Leu-7	11.0	3.5	6.0 - 16.0
Leu-11b	9.9	1.5	1.0 - 30.0
OKT3	60.6	5.4	15.0 - 88.0
OKT4	32.9	2.9	7.0 - 52.0
OKT8	29.4	3.3	7.0 - 62.0
B1	9.6	1.7	0.8 - 24.0

* >98% were mononuclear cells

intravenous injection of hydrocortisone the symptoms disappeared within 30 min. At all the other treatment times the cutaneous tests were negative and no allergic reactions were noted. No other side-effects related to therapy except for mild fever and shivering were seen (Table 3).

The dosage schedules and clinical effects are shown in Table 3. Two patients (nos: 3,4) had a tumor reduction. In pat. 3 there was a dramatic decrease in serum CA 19-9 concentration after the second course of Moab (Fig 1).

This was accompanied by a diminishing in size of the single tumor lesion in the liver parenchyma from 5 cm in diameter to 2 cm. Interestingly, at that particular infusion time the highest number of AMC (2.2×10^{10}) containing a large number of OKM1⁺ cells (surface determinant on monocytes/macrophages and NK-cells) (Table 4) was isolated and given back to the patient. Most probably the majority of the cells were monocytes/macrophages as cells expressing Leu-11b

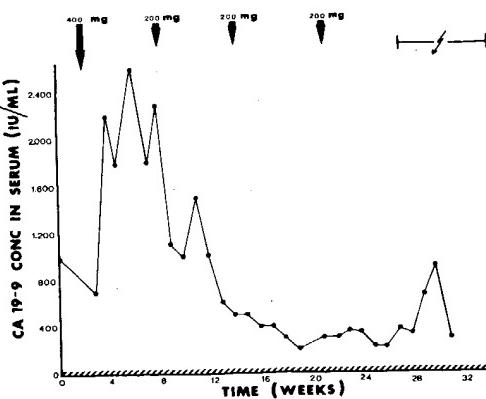


FIG. 1. Variations in serum CA 19-9 conc. in pat. no 3 during therapy. Arrows indicate Moab infusion times. Shaded area normal serum levels of CA 19-9.

TABLE 3. DOSAGE SCHEDULES AND CLINICAL EFFECTS

Pat. no	Date of adminis- tered Moab	Amount of Moab (mg)	Side effects	Treat- ments after Moab	Time of follow up from start of therapy	Status** at follow up
1.	01/17/85 02/28/85	400 200	None None	Chemo- therapy	1 mo 3 mo 06/26/85	Prog. Prog. Dead
2.	02/14/85 03/28/85	400 200	None Palmitch	Chemo- therapy	1 mo 3 mo 07/24/85	Prog. Prog. Dead
3.	05/02/85 06/13/85 07/25/85 09/12/85	400 200 200 200	None None None None	Radio- therapy and chemo- therapy	1 mo 3 mo 7 mo 10 mo	Stable Regr. Prog. Prog.
4.	05/23/85 07/17/85 09/09/85 11/24/85	400 200 200 200	None None None None	None	1 mo 3 mo 7 mo 10 mo	Stable Regr. Stable Stable
5.	06/12/85 07/24/85 09/11/85	400 200 200	None None None	Radio- therapy	1 mo 3 mo 6 mo 9 mo	Stable Stable Prog. Prog.
6.	06/05/85 07/18/85 09/05/85	400 200 200	None None None	Chemo- therapy	1 mo 3 mo 6 mo 9 mo	Stable Prog. Prog. Stable
7.	09/18/85 11/06/85 01/14/85	400 200 200	None Shivering None	None	1 mo 3 mo 6 mo	Stable Stable Prog.
8.	09/19/85 11/04/85 12/19/85	400 200 20	Fever Fever Anaphy- laxia	None	1 mo 3 mo 6 mo	Stable Stable Stable

* When other treatments were instituted the patient was regarded as a failure.
 ** Disease status is related to the preceding judgement.

were low. Pat. no 4 had histologically proven metastases to mesenteric lymph nodes, visualized by ultrasonic examination. Serum levels of CEA and CA 19-9 were normal as were liver function tests. The enlarged lymph node conglomerate decreased in size during treatment from 5 cm of the largest diameter to 1-2 cm. Presently, the patient has no clinical evidence of disease.

In four patients (no:s 5,6,7,8) a stable disease was recorded from three to six months on. (A stable disease was defined as no change in serum tumor markers, liver function tests and in the size of tumor lesions respectively.) In pat.s 1 and 2 there was a continuous progression of the disease. These two patients had the largest tumor masses.

In no patient serum complement consumption could be seen as exemplified in Fig 2.

INICAL EFFECTS

	*	Time of follow up from start of therapy	Status ** at follow up
y	1 mo 3 mo 06/26/85	Prog. Prog. Dead	
y	1 mo 3 mo 07/24/85	Prog. Prog. Dead	
y	1 mo 3 mo 7 mo 10 mo	Stable Regr. Prog. Prog.	
y	1 mo 3 mo 7 mo 10 mo	Stable Regr. Stable Stable	
	1 mo 3 mo 6 mo 9 mo	Stable Stable Prog. Prog.	
	1 mo 3 mo 6 mo 9 mo	Stable Prog. Prog. Stable	
	1 mo 3 mo 6 mo	Stable Stable Prog.	
	1 mo 3 mo 6 mo	Stable Stable Stable	

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be seen as exemplified in

TABLE 4. CELL SUBPOPULATIONS (%) OF ISOLATED AMC IN PAT. NO 3 AT THE SECOND TREATMENT TIME

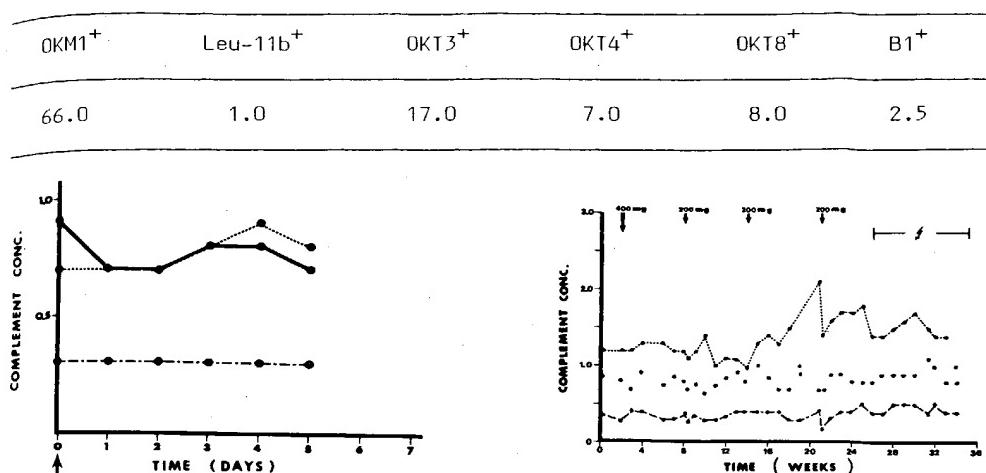


FIG 2. Variations in serum complement concentrations. Following one week after Moab 17-1A infusion in pat. no 9 (to the left) (pat. no 9 was recently started on therapy and is not included in Tables 1 and 3) and during a prolonged observation period in pat. no 3 (to the right). Arrows indicate Moab infusion C3 (—), C3d (.....), C4 (----).

If a tumor lesion was easily accessible for thick needle biopsy, a biopsy was performed before Moab infusion and after 24 h. Totally, 32 biopsies have been done. The 19-9A and 19-9B as well as the 73-3 Moabs were detectable in B5 fixed and paraffin embedded material. In contrast, 17-1A was only detectable in frozen sections. The Moab 17-1A stained the cytoplasm of the tumor cells with moderate intensity sometimes accentuated along the basement membrane where the tumor glands were evident. In most biopsies about 90% of the tumor cells were stained. No antigenic modulation was seen after treatment. The results with Moab 19-9A and 19-9B respectively were similar. A strong cytoplasmic staining was seen in approximately 10-80% of tumor cells in most biopsies. Staining was also found in the lumen and along the apical surface of the cells suggesting that the antigen is present on a secreted product. The 73-3 had a moderately strong cytoplasmic staining which was consistently accentuated along the basement membrane.

The potential influence of the treatment with Moab-armed monocytes/macrophages on the number and type of leucocytes infiltrating in and around the metastasis was estimated immunohistochemically. The evaluation was to some extent restrained by the limited amount of viable tumor material. Furthermore, the Moabs Leu M-1 and Leu-11b showed cross reactivity with tumor cells hampering the identification of positive cells. Leu M-1 is also reactive against endothelial cells which may also interfere with the interpretation of the staining. Comparing the pre- and posttreatment biopsies, no dramatic differences in the number of infiltrating cells were seen. However, occasionally there was an increase in the number of Leu M-1 and Leu-11b positive cells. T-lymphocyte numbers seemed to remain stable although sometimes a slight decrease was seen. Leu M-5 and to some extent Leu M-3 stained a cell population just beneath the basement membrane. These cells seemed to be elongated and may therefore be of fibroblastic/reticular type. In 5 out of 6 analysed post treatment biopsies, mouse IgG could be detected, most prominent along the basement membrane. In the corresponding pretreatment biopsies three cases were negative, in two the material was insufficient and in one a very weak staining was observed. The results suggest that the infused Moab 17-1A is "homing" to the basement membrane of the tumor glands. A faint and focal C3 positivity was sometimes seen in some of the



FIG. 3
in IgG
Arrows

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tumor cells. No visible basement membrane staining was found with the anti-C3 antibody. There was no difference in the C3 staining between the pre- and post-treatment biopsies. Examples of immunohistochemical analyses from one pat (no 5) are shown in Table 5.

All patients developed antibodies against Moab 17-1A. The anti-mouse antibody titers increased after every infusion. The IgM antibody levels were low. As the IgG anti-mouse antibody titers increased the shorter became the half-life time of the infused Moab 17-1A. Variations in the antibody levels in relation to Moab 17-1A infusions are exemplified by pat. no 3 shown in Fig. 3.

DISCUSSION

No effective chemotherapy regimen with regard to survival exists for the treatment of patients with metastasizing colo-rectal cancer. Complete and partial remissions may however be achieved. Thus, there are a need for other treatment modalities to be tested.

The development of the hybridoma technology has made it possible to produce antibodies of predefined specificity in large quantities. Such antibodies can

TABLE 5. ANTIGENIC EXPRESSION, MONONUCLEAR CELL INFILTRATION, MOUSE-IgG AND COMPLEMENT DEPOSITION RESP. IN TUMOR BIOPSIES FROM PAT. NO 5 DURING THERAPY.

		1st treatment before	1st treatment after	2nd treatment before	2nd treatment after	3rd treatment before	3rd treatment after
17-1A*			+++	+++	+++	+++	+++
19-9 A&B*			+++	+	++	+	+
73-3*			+++	+++	+++	+++	+++
Cytok.*			+++	+++	+++	+++	+++
Cellular infiltration**	S	11	12	8	10	22	6
Leu-4**	e		7	NT	NT	13	4
Leu-3**	c		6	11	7	7	5
Leu-2**	r		4	10	4	13	<1
Leu M-1**	o		3	<1	14	9	5
Leu M-3**	m		15	20	12	6	6
Leu M-5**	u		6	23	15	10	8
Leu-11b**	t		2	3	11	5	3
C3§	N			NT	-	-	+
Mouse IgG§			(+)	-	-	-	(+)

NT = not tested (material insufficient).

* Frequency of stained tumor cells +++ > 75%, ++ 50-75%, + < 50%.

** The mean number of infiltrating mononuclear leucocytes in and around the tumor in ten HPFs.

§ - negative; +- very weak; (+) weak; + moderate.

as found with the anti-C3 Ig between the pre- and post-analyses from one pat. (no 5).

7-1A. The anti-mouse anti-Ig antibody levels were low, shorter became the half-life of the antibody levels in relation to no 3 shown in Fig. 3.

Survival exists for the treated cancer. Complete and partial responses are needed for other treatment.

made it possible to produce antibodies. Such antibodies can

INFILTRATION, MOUSE-IgG IN BIOPSIES FROM PAT. NO 5

	3rd treatment before	after
+++	+++	
+	+	
+++	+++	
+++	+++	
22	6	
13	4	
7	5	
13	<1	
9	5	
6	6	
10	8	
5	3	
+	(+)	
-	(+)	

%, + < 50%.

Biopsies in and around the tumor

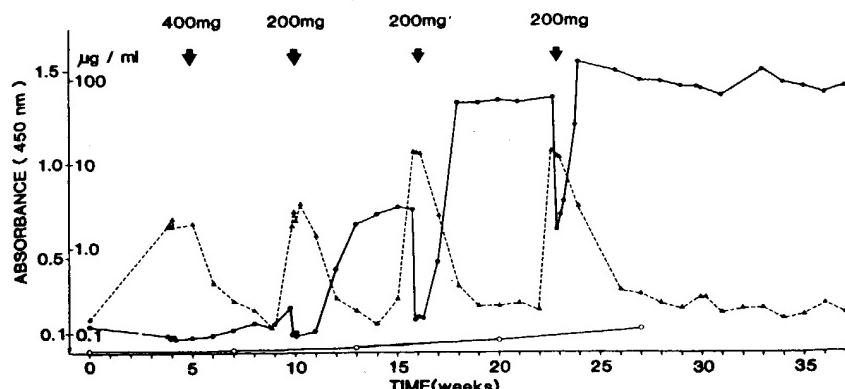


FIG. 3. Variations in Moab 17-1A (—○—) conc. ($\mu\text{g}/\text{ml}$) in serum in pat. no 3 and in IgG (—●—) and IgM (○—○) antibody levels (abs.) resp. against Moab 17-1A. Arrows indicate Moab infusions.

be produced against TAA and used for therapy of tumor patients. 17-1A, a protein antigen, present on the surface of colo-rectal cancer cells has been used by prof. H. Koprowski as an immunizing agent to produce Moab of sub-class IgG_{2A} for therapy. These antibodies are used by us in a pilot study to establish the therapeutic efficacy of Moab per se. It is important to evaluate this treatment principle alone before studies are initiated where Moab is used in combination with other agents as example, carriers of toxins, cytostatics or radionucleoids.

Several mechanisms of the immune system may be responsible for the destruction of tumor cells mediated by Moab. One of these may be lysis by cytotoxic monocytes/macrophages or NK-cells bound via their Fc-receptors to the Fc-part of the monoclonal antibody molecule. In experimental studies (in vitro and in vivo) this effector mechanism seems to be of importance and both subpopulations may be operating [9,4,6,7,8]. In the treatment of patients with solid tumors a better therapeutic effect was seen when Moab was given together with isolated peripheral blood mononuclear cells preincubated in vitro with the monoclonal antibodies than when Moab was administered alone (Douillard, personal communication). These background informations were the rationals for our treatment protocol, designed with the intention to increase/direct cytotoxic cells to the tumor lesions and/or increase the functional activity by pre-incubation in vitro. With our technique for isolation of 8×10^6 cells were obtained and about 30% belonged to the effector cell population participating in the ADCC reaction (monocytes and NK-cells) (Table 2). A corresponding number of cells were labelled with Moab 17-1A after 1 h incubation in vitro. By FACS analysis we could show that both monocytes and lymphocytes were armed with Moab (data not shown). However, at the moment we have no proof that the isolated cells have been directed to the tumor lesions. 24 h after reinfusion of the AMC no increase in cytotoxic cells were noted (Table 5). However, we have recently changed our follow-up protocol and performed the biopsy 72 h or 96 h after the infusion as Douillard has shown in an experimental system that when Moab was administered together with isolated peripheral mononuclear cells maximal infiltration could be seen on days 3 to 6. Furthermore, we are also performing experiments where we label the isolated cells with indium ¹¹¹ for making tracer studies. From the biopsy studies it could also be seen that after Moab infusions no antigenic modulation occurred and small amounts of mouse-IgG were attached to the tumor cell surface but no complement.

In two patients an objective tumor reduction was seen. Two patients had a continuous progression of the disease after institution of Moab therapy. These two patients had the largest tumor volume. In further four patients a stable disease was noted varying between 3 and 6 months on from start of therapy.

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Whether this stabilization is related to therapy can not be settled. In other pilot studies using Moab 17-1A in the treatment of patients with colo-rectal cancer it has been shown that patients "responding" to therapy survived longer than those who did not "respond". A few patients entered a partial remission and some had a "stabilization of the disease" (Douillard, personal communication). Good responses have been seen by Sears et al⁹ showing disappearance of detectable disease in 3 out of 20 patients. An interesting study is presently carried out in Nantes where patients after surgery of Dukes B2 and C colo-rectal cancer receive Moab 17-1A as adjuvant therapy with the aim to cope with micro-metastases.

The side effects of repeated infusions were neglectable with one exception. All patients were tested intracutaneously before administration of Moab. At the third infusion time in pat. no 8 an immediate anaphylactoid reaction occurred. In this patient the cutaneous test was misread as negative but was obviously positive. In another pat. no 2 a positive reaction was also seen but allergic side-effects were probably prevented by pre-treatment with hydrocortisone. At all other 22 treatment times the tests were negative. Thus, intracutaneous testing seems to be a safe procedure to predict allergic side-effects against the infused Moab. All the patients developed IgG and IgM anti-mouse antibodies but this fact did not seem to negatively influence the appearance of adverse effects. However, the half-life on infused Moab decreased by increasing anti-mouse antibody titers.

This preliminary progress report shows that repeated Moab infusions can be given safely. Tumor reduction can be seen after infusion of Moab. However, much has to be done to better understand the mechanisms behind the tumor destruction with the aim to develop strategies by which cell death can be enhanced.

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